BioRisk

BioRisk 23: 17–43 (2025)
DOI: 10.3897/biorisk.23.151538

Research Article

The role of grooming in regulating biomass growth of ants and symbiotic fungi under entomopathogenic fungal infection: experiments and mathematical modelling

Isabella Bueno[1], João Vitor Mendes Ferraz de Toledo[1], Lucas Santos Canuto[1], Wesley Augusto Conde Godoy[1]

1 *Laboratory of Ecology and Forest Entomology, Department of Entomology and Acarology, Luiz de Queiroz College of Agriculture, University of São Paulo, Av. Pádua Dias, 11, São Judas, Piracicaba, São Paulo, Brazil*

Corresponding author: Wesley Augusto Conde Godoy (wacgodoy@usp.br)

Abstract

This study presents an extended mathematical model, originally developed to explore biomass dynamics in *Acromyrmex* ants and their symbiotic fungus, which has been adapted to investigate biomass growth in *Atta sexdens* and its fungal partner. The model incorporates ant grooming as a defence against entomopathogenic fungi, building on experimental data where self-grooming and allogrooming were quantified across three groups: *Metarhizium anisopliae*, *Escovopsis phialicopiosa*, and a control. A second chamber was introduced to simulate biomass transfer between compartments. Inflection points in growth curves were identified to detect shifts in population dynamics, and bifurcation diagrams explored key parameters affecting system stability, namely worker allocation to fungal cultivation, ant mortality, and fungal mortality. *Metarhizium anisopliae* significantly reduced both ant and fungal biomass, even under optimal grooming conditions, owing to its direct virulence to workers. In contrast, *E. phialicopiosa*, an opportunistic pathogen, had minimal impact unless fungal mortality exceeded a critical threshold. Self-grooming proved more effective than allogrooming in mitigating *M. anisopliae* effects, likely owing to prioritisation of individual defences under high pathogen pressure. Spatial dynamics enhanced resilience: one-way transfer between chambers redistributed biomass, delaying inflection points and bolstering structural stability. Bifurcation analysis revealed that extreme proportions of workers cultivating the symbiotic fungus reduced the biomass for both partners, whilst ant and fungal mortality rates led to non-linear declines in most simulations. These findings underscore the role of multi-chamber architecture in mitigating pathogen impacts in *Atta* colonies and suggest potential applications for biological control strategies by identifying behavioural and structural factors that may limit or enhance the effectiveness of pathogenic fungi in field settings. The model provides a useful framework for understanding epidemiological dynamics in natural nests, integrating behavioural defences and spatial strategies to safeguard this mutualism.

Key words: Allogrooming, ant-fungus mutualism, interactions, leaf-cutting ants, self-grooming

OPEN ACCESS

Academic editor: Pavel Stoev
Received: 27 February 2025
Accepted: 13 May 2025
Published: 11 June 2025

Citation: Bueno I, Toledo JVMFde, Canuto LS, Godoy WAC (2025) The role of grooming in regulating biomass growth of ants and symbiotic fungi under entomopathogenic fungal infection: experiments and mathematical modelling. BioRisk 23: 17–43. https://doi.org/10.3897/biorisk.23.151538

Copyright: © Isabella Bueno et al.
This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0).

Introduction

The symbiosis between Attina ants and Basidiomycota fungi constitutes an obligate mutualism: ants provide substrates and protect the fungi from pathogens and parasites, while the fungi serve as the sole food source for larvae and a significant component of the workers' diet (Weber 1966; Mehdiabadi and Schultz 2010; Hölldobler and Wilson 2011). This specialised relationship, approximately 66 million years old (Schultz et al. 2024), involves a complex network of microorganisms, including yeasts, viruses, and bacteria, whose roles remain largely unexplored (Currie et al. 1999). Dense, genetically uniform colonies render ants vulnerable to pathogenic microbes (Schmid-Hempel 1998; Rissanen et al. 2022). To counter this, they have evolved a multi-layered defence system, integrating physiological responses (Fernández-Marín et al. 2006) with behavioural mechanisms such as self-grooming, allogrooming, and cleaning of brood and fungal gardens (Currie et al. 1999; Goes et al. 2022).

Leaf-cutting ants of the genus *Atta* are among the most important agricultural pests in Latin America. Members of this genus occur from the southern United States to Argentina (Mariconi 1970; Delabie et al. 2011; Forti et al. 2020). Their ecological significance is reflected in their status as dominant herbivores in Neotropical biomes (Bucher 1982; Costa et al. 2008), with the potential to consume up to 17% of the available foliar biomass (Costa et al. 2008). From an economic perspective, *Atta* species cause substantial damage to forest plantations. In *Eucalyptus* spp., sustained defoliation reduces productivity (Zanetti et al. 2003), while in *Pinus* spp., it negatively affects tree growth (Scherf et al. 2022). In agricultural systems, severe infestations can lead to considerable yield losses in crops such as cassava (*Manihot esculenta*) and coffee (*Coffea arabica*) (Bertorelli et al. 2006; Varón et al. 2007).

In pastures, intensive removal of plant biomass limits forage availability (Fowler 1986). The combination of extensive distribution, highly efficient foraging, and broad host adaptability consolidates *Atta* species as a major challenge for agricultural and forestry management in the Neotropical region. These impacts underscore the economic importance of understanding the ecological dynamics of *Atta* colonies and the need for effective management strategies. In addition to physiological and behavioural defences, fungus-farming ants employ spatial strategies to minimise pathogen transmission.

Age- and caste-based spatial segregation exemplifies this approach (Wilson 1980), whilst controlled movement between functional groups and waste segregation further reduce disease spread (Farji-Brener and Tadey 2012), enhancing organisational immunity. In the genus *Atta*, an intricate network of underground chambers (Moreira et al. 2010) plays a pivotal role in collective immunity, bolstering colony resilience. The chamber expansion follows a unidirectional pattern during colony development. Studies show that, at the time of expansion, *Atta* workers relocate the brood and fungal biomass to locations where new chambers will be excavated and established (Römer and Roces 2014). This directional flow of resources reflects the colony's developmental trajectory, in which older chambers act as sources of biomass for new compartments. This compartmentalised structure, dedicated to fungal cultivation, brood rearing, and waste disposal, limits pathogen dissemination (Hart and Ratnieks 2002), rendering these ants particularly resistant to biological control strategies.

A key challenge in biological control is the structural complexity of nests, since interconnected chambers hinder the uniform application of biocontrol agents and the empirical study of disease dynamics. Typically, control strategies involving entomopathogenic fungi, such as *Metarhizium anisopliae*, are successful when the fungi infect insects through spores that adhere to the insect's surface, germinating and penetrating the cuticle to initiate an infectious process. Given the complexity of these social immunity mechanisms, analysing these systems poses a challenge, highlighting key considerations for using entomopathogenic fungi in biocontrol.

Mathematical models of organism interactions are vital in ecology for describing and predicting population dynamics within complex systems under diverse scenarios (Lima et al. 2009). Mutualistic or symbiotic relationships, such as those between ants and fungi in compartmentalised systems, are widely studied (Dejean et al. 2023). The relationship between fungus-cultivating ants and their fungi offers a unique opportunity to explore population dynamics and the factors influencing stability and growth under varying environmental conditions.

This study employed a mathematical model to investigate the dynamics between leaf-cutting ants and their symbiotic fungus within a two-chamber system, where one-way transfer between compartments is density-dependent and follows a logistic function. This configuration enables the examination of critical factors, including carrying capacity, the influence of external stressors (such as pathogens), and ant behavioural responses to population growth. Furthermore, it facilitates modelling the effects of self-care and social hygiene, specifically self-grooming and allogrooming, in response to pathogenic fungal spread, enhancing our understanding of how these behaviours mitigate disease impacts. The model extends the framework proposed by Kang et al. (2011), originally developed to describe biomass growth dynamics in *Acromyrmex* ants and their symbiotic fungus.

The present study addressed fundamental ecological questions regarding how hygienic behaviours, such as self- and allogrooming, contribute to the resilience of leaf-cutting ant colonies and their fungal symbionts when faced with pathogenic fungal infections. Specifically, we investigated how the addition of an extra nest chamber, a structural feature typical of the genus *Atta*, could mitigate the impact of pathogens, what critical mortality thresholds compromised mutualistic stability, and how worker allocation and behavioural strategies shaped the population dynamics of both the ants and their fungal gardens.

These questions are central to the development of effective biological control strategies, given that the structural complexity of subterranean nests and the behavioural defences of these ants present significant challenges to the application of pathogens under field conditions. By integrating experimental data with mathematical modelling, this study aimed not only to advance our ecological understanding of the ant-fungus mutualism but also to identify key parameters (e.g., optimal proportion of workers allocated to fungal cultivation, one-way transfer rates between chambers) that can inform more targeted interventions, minimising environmental impacts while optimising population control in regions where this genus is considered an agricultural pest.

Methods

Data Collection: fungal suspension preparation

We collected incipient *Atta sexdens* colonies on the campus of the Federal University of São Carlos (UFSCar), in Araras, São Paulo, with the material kindly provided by the university, and maintained them under controlled laboratory conditions (25 ± 1 °C, 70% relative humidity, and a 12-hour light/dark cycle). Prior to the experiments, we transported the colonies to the Luiz de Queiroz College of Agriculture (ESALQ) in Piracicaba, São Paulo, where we conducted all the experiments. At the time of the experiments, the colonies, maintained for approximately two years, had fungal garden chambers with a volume of approximately 2 litres. We prepared fungal suspensions of *Metarhizium anisopliae* strain E9 (sourced from the ESALQ-USP collection) and *Escovopsis phialicopiosa* strain LESF 021 (sourced from the UNESP Rio Claro collection) by culturing the fungal strains on potato dextrose agar (PDA) at 25 °C for 4–5 days. Following this incubation, we scraped the conidia from the cultures, resuspended them in a 0.05% Tween 80 solution, and adjusted this to a final concentration of 10^8 conidia/ml. Once the fungal preparations were completed, we initiated the experiments focusing on the contamination of ant colonies.

Experimental treatments to estimate the time ants spent on self-grooming and allogrooming

We conducted the experiment using miniaturised colonies. We housed workers from four source colonies in plaster-lined Petri dishes containing fragments of symbiotic fungal gardens (0.5 g), one larva, one pupa, and ten medium-sized ants, and allowed them to acclimate for 48 hours. Treatments comprised T1: Tween 80 control; T2: *Metarhizium anisopliae* suspension; and T3: *Escovopsis phialicopiosa* suspension. Suspensions were applied using a 500 µl airbrush spray. The experiment followed a randomised block design, with four colony-based replicates per treatment. We recorded the ant behaviour (4 hours per replicate) using a vertically positioned Sony Handycam under controlled conditions (23 °C, 60% relative humidity). We made observations at 30-minute intervals, with 5-minute focal sampling analysed using BORIS v7.13.8. The time spent on self-grooming or allogrooming was measured in seconds for the three treatments specified above. Post-treatment, we maintained the ants under controlled conditions (25 °C, 70% relative humidity), monitoring the mortality and fungal sporulation daily.

Revisiting Kang's model

This study builds on a previous investigation that proposed a theoretical framework to model the ant population of the genus *Acromyrmex*. The model, developed by Kang et al. (2011), comprises two differential equations designed to explore the population dynamics of ants and their symbiotic fungi. These differential equations describe the temporal changes in ant biomass ($\frac{dA}{dt}$) and symbiotic fungal biomass ($\frac{dF}{dt}$) as functions of growth and mortality rates for both species, as well as their mutual interactions.

Kang et al. (2011) outlined the following assumptions regarding the interactions between workers and the fungus during the initial stages of colony devel-

opment: (I) Each worker assigns a fixed portion of its energy to tasks inside and outside the colony, and therefore, only a proportion of the colony cares for the fungal gardens, queen, and larvae. (II) The ant colony increases as the queen, larvae, and adult ants feed on the fungus. The ants' feeding pattern is modelled using the Holling Type I approach, where the fungal biomass *F* is multiplied by a constant growth rate r_a. Additionally, ant mortality depends on their population density (Holland and DeAngelis 2010). Consequently, the changes in the ant population over time can be mathematically outlined with this equation:

$$\frac{dA}{dt} = (r_a F - d_a A)\, A \tag{1}$$

Thus, r_a represents the parameter estimating the maximum growth rate of ants, while d_a denotes their mortality rate. (III) Regarding the fungus, there are a few important considerations: First, their growth depends on the harvesting and processing of leaves, which in turn depends on the allocation of labour (*a*); second, the fungus exhibits a type III numerical response to the ants, with a saturation constant (*b*) (Real 1977); and finally, they are assumed to face density-dependent mortality (d_f), as well as a consumption rate by the ants (*c*) (Holland and DeAngelis 2010). Accordingly, the final equation for the fungus is as follows:

$$\frac{dF}{dt} = \left(\frac{r_f a A^2}{b + aA^2} - d_f F - r_a c A \right) F \tag{2}$$

Mathematical and computational methodology for the fungus-insect model in two chambers

Equations (1) and (2) from the model by Kang et al. (2011) were extended here to investigate specific questions, such as colony architecture and the defence of ants and their symbiotic fungus against pathogenic fungi. The study by Kang et al. (2011) was originally designed to evaluate biomass changes in *Acromyrmex*, a genus of leaf-cutting ants that includes some species which cultivate their symbiotic fungus in a single chamber (Kang et al. 2011). However, the species examined here, *Atta sexdens*, cultivates its symbiotic fungus in multiple chambers. Consequently, we structured the current model to analyse the system using two chambers. Although *Atta* colonies typically construct multi-chamber systems, we implemented a simplified two-chamber model to streamline the algebraic structure and gain a basic understanding of the system.

The first step was to develop a representation of the defensive mechanisms that ants have against pathogenic fungi, i.e., self-grooming and allogrooming. Next, the chambers represent patches connected to allow the transfer of ants and the symbiotic fungus between them. The key criterion governing the movement of organisms between chambers was the carrying capacity of each chamber. In this approach, chamber *A* has a spatial limit for both the ants and the symbiotic fungus. A one-way transfer of individuals occurs from chamber *A* to chamber *B* when populations in *A* reach saturation, based on resource availability for subsistence. Regarding colony defences against pathogenic fungi, we incorporated estimates of the time spent on self-grooming and allogrooming by workers. We assumed that the longer the time spent grooming, the greater the defence, a well-documented assumption (Hughes et al. 2002; Fernández-Marín et al. 2006).

We developed an R code to implement the system, providing a computational approach to modelling the interactions between ant biomass and fungal biomass within a system of two connected chambers. We used a set of differential equations to describe these dynamics, incorporating growth, mortality, one-way transfer between chambers, and pathogenic influences. We employed the deSolve package in R (Soetaert et al. 2010) to solve this system with the lsoda method, enabling simulations of interactions across the chambers.

Mathematical representation of self-grooming and allogrooming

We modelled the effect of self-grooming on the mortality rates of ants and the symbiotic fungus by considering specific mortality rates of ants (d_a (pathogenic)) and fungi (d_f (pathogenic)) due to the pathogenic fungi *M. anisopliae* and *E. phialicopiosa*. These rates were calculated as:

$$d_{a(pat)} = k_a \left(1 - \frac{s_t}{s_{tmax}}\right) \quad (3)$$

$$d_{f(pat)} = k_f \left(1 - \frac{s_t}{s_{tmax}}\right) \quad (4)$$

$$\text{where: } \begin{cases} s_{t\max} > 0; \\ \frac{s_t}{s_{t\max}} \leq 1; \\ s_t \leq s_{t\max} \end{cases}$$

Components k_a and k_f are the intensities of the pathogenic fungi effect on ants and the symbiotic fungus, respectively. The term $\left(1 - \frac{s_t}{s_{tmax}}\right)$ represents the reduction in pathogen-induced mortality due to self-grooming. As s_t increases, the mortality rates decrease, reflecting the protective effect of grooming. Equations (9) and (10) were structured to model the allogrooming as well. The transcription of this may be represented simply by replacing s_t with a_t. Finally, the total mortality of the ants and the symbiotic fungus in each chamber is the sum of the baseline mortality rates and the pathogen-induced mortality rates:

$$d_{aA} = d_a + d_{apat\ A}, \quad (5)$$

$$d_{fA} = d_f + d_{fpat\ A} \quad (6)$$

where d_a and d_f are the baseline mortality rates for the ants and the symbiotic fungus. The same equations implemented for chamber *B* are written as:

$$d_{aB} = d_a + d_{apat\ B}, \quad (7)$$

$$d_{fB} = d_f + d_{apat\ B} \quad (8)$$

Definition of the mathematical model

Differential equations for two connected chambers describe the populations of ants and their symbiotic fungus. This requires considering the transition between chambers *A* and *B* and that this transition will be unidirectional, representing how the ants move the fungal garden to new chambers as the first chamber begins to reach its limit. The one-way transfer is represented by the following equation:

$$\begin{cases} \text{If } F_A > F_{\max}; \begin{cases} D_A = D * A_A * \left(1 - \frac{A_B}{K_B}\right) \\ D_F = D * (F_A - F_{\max}) \end{cases} \\ \text{else; } D_A = 0 \text{ and } D_F = 0 \end{cases} \tag{9}$$

These equations model the rates of change in ant and fungal populations based on resource-dependent growth influenced by interspecific interactions while accounting for mortality, including a general death rate (Kang et al. 2011) and specific effects of pathogens as modulated by self-grooming and allogrooming. With this information in mind, the final equations for biomass growth dynamics in chambers *A* and *B* are:

Chamber *A*:

$$\frac{dA_A}{dt} = \left(r_a F_a - d_a A_A\right) A_A - D_A \tag{10}$$

$$\frac{dF_A}{dt} = \left(r_f \frac{a_A A_A^2}{b + a_A A_A^2} - d_f F_A - r_a r_c A_A\right) F_A - D_F \tag{11}$$

Chamber *B*:

$$\frac{dA_B}{dt} = \left(r_a F_b - d_a A_B\right) A_B + D_A \tag{12}$$

$$\frac{dF_B}{dt} = \left(r_f \frac{a_B A_B^2}{b + a_B A_B^2} - d_f F_B - r_a r_c A_B\right) F_B + D_F \tag{13}$$

These equations differ from those in Kang et al. (2011) primarily by adding a second chamber and including protective mechanisms for the colony, represented by self-grooming and allogrooming. A combination of Kang's model parameters and those estimated in the laboratory was used in the extended model, as described in Table 1.

Table 1. Parameter values used in the mathematical model.

Parameter	Description	Value	Source	Unit
ra	Growth rate of ants	0.1	Kang et al.*	day^{-1}
rf	Growth rate of symbiotic fungus	0.7	Kang et al.	day^{-1}
c	Fungus-to-ant biomass conversion rate	0.0045	Kang et al.	
da	Mortality rate of ants	0.1	Kang et al.	day^{-1}
df	Mortality rate of symbiotic fungus	0.2	Kang et al.	day^{-1}
ka	Pathogen effect intensity on ants	0.15	Experimental	
kf	Pathogen effect intensity on symbiotic fungus	0.15	Experimental	
b	Half-saturation constant for fungal growth	0.002	Kang et al.	
p	Proportion of workers in colony	1	Kang et al.	
q	Proportion of workers allocated to fungus cultivation	0.5	Experimental	
D	One-way transfer rate (A→B)	0.1	Experimental	day^{-1}
st	Time spent on self-grooming (see Table 2)	Variable	Experimental	seconds
at	Time spent on allogrooming (see Table 2)	Variable	Experimental	seconds

*Kang et al. (2011).

Inflection points

The model evaluated the inflection points for the ants and fungal biomasses in each chamber. These points are critical for understanding system dynamics, as they mark the transition in biomass change where concavity shifts from

accelerating to decelerating or vice versa. Inflection points were identified by analysing the second derivative of biomass curves, marking the most pronounced shifts in growth or decline.

Bifurcation diagrams

Bifurcation theory is a mathematical framework used to study qualitative and quantitative changes in the behaviour of dynamical systems as their parameters vary. In differential equations, a bifurcation occurs when a small change in a system's parameters induces a sudden topological shift in its dynamics, such as the emergence of new equilibrium points, the disappearance of existing ones, or the onset of oscillations. Consider a system of ordinary differential equations (ODEs) of the form:

$$\frac{dx}{dt} = f(x, p)$$

where x represents the state variables (e.g., the biomasses of ants and the symbiotic fungus), p denotes the system parameters (e.g., q, d_a, d_f), and f is a smooth function describing the system's dynamics in the ant-fungus system.

In this study, we used bifurcation theory to explore the parametric space of: q (the proportion of ants cultivating the symbiotic fungus), d_a (the baseline mortality rate of ants), and d_f (the baseline mortality rate of the symbiotic fungus), examining how self-grooming (s_t) and allogrooming (a_t) influence these parameters and affect the system's stability and dynamics. We selected these three parameters based on a prior sensitivity analysis with all model parameters. When evaluated for self-grooming and allogrooming, the criterion for choosing these parameters was their significant impact on the biomasses of the ants and the symbiotic fungus. Critical thresholds can be identified by examining bifurcation diagrams for these parameters, enabling predictions of how the system responds to ecological perturbations, such as variations in pathogen pressure or grooming behaviour.

The bifurcation diagram is essential for understanding how variations in the time spent on self-grooming (s_t) and allogrooming (a_t) influence the dynamics of the ant-symbiotic fungus system. Specifically, s_t and a_t modulate the pathogen-induced mortality rates of ants and the symbiotic fungus, as described by the terms in equations (3) and (4). These terms, in turn, directly affect the total mortality rates presented in equations (5) to (8), thereby shaping the system's stability. We conducted the simulations using the R packages ggplot2, patchwork, and deSolve (R codes:10.5281/zenodo.15364469). We used the lsoda method to numerically solve the differential equations. For each simulation, we extracted and plotted the final values of the time series to represent the system's equilibrium states in the bifurcation diagrams.

Results

Ant responses to the pathogens varied depending on the grooming type (Table 2). Table 2 presents laboratory estimates of the time spent on self-grooming and allogrooming for the control, *M. anisopliae*, and *E. phialicopiosa* treatments. The largest difference among treatments (T1, T2, T3) was between the maximum and mean allogrooming values (Table 2).

Table 2. Duration of self-grooming and allogrooming (seconds). *

Self-grooming			
Value	T1 (control)	T2 (*M. anisopliae*)	T3 (*E. phialicopiosa*)
Maximum	168	114	185
Mean	14.4	12	14
Minimum	0.30	0.53	0.73
Allogrooming			
Value	T1 (control)	T2 (*M. anisopliae*)	T3 (*E. phialicopiosa*)
Maximum	1701	169	1639
Mean	330	54	82
Minimum	1.50	3.87	1.23

* Raw data are available in the Suppl. material 1.

Fig. 1A depicts the ant and fungal populations according to the model of Kang et al. (2011), showing sigmoid growth. Fig. 1B includes a one-way transfer from chamber *A* to *B*, with biomasses decreasing in *A* and increasing in B. Fig. 2 illustrates maximum self-grooming, with inflection points (solid blue circles for chamber A and solid red circles for chamber B) in biomass curves for T1, T2, and T3, reflecting critical growth shifts. In T2 (*M. anisopliae*), the ant and fungal biomasses decreased compared to T1 (control), with inflection points delayed. T3 (*E. phialicopiosa*) closely resembled T1, with minor shifts in inflection values (Fig. 2). Figs 3, 4 show mean and minimum self-grooming, respectively, and curves exhibiting analogous trends with slight differences in their inflection points.

Metarhizium anisopliae (T2), even exposed to maximum allogrooming, significantly reduced the biomasses for both ants and the symbiotic fungus compared to T1 (Fig. 5), with inflection points shifting to higher values, resulting in a time-delayed effect, except in Chamber A. T3 mirrored T1, with slight differences in inflection point values. The mean and minimum times spent on allogrooming produced similar growth curves across the treatments, with minor

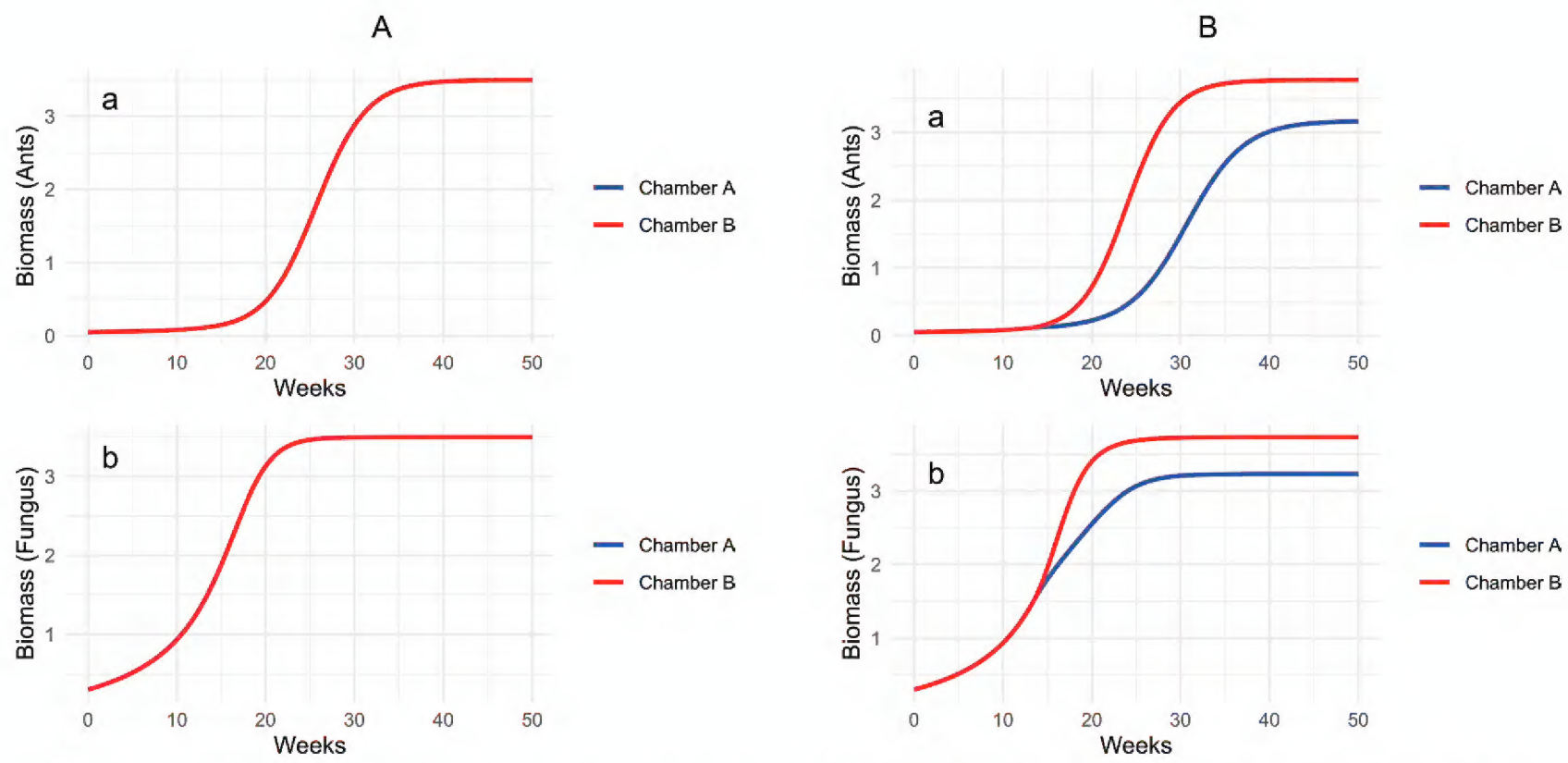

Figure 1. Biomass of leaf-cutting ants (a) and symbiotic fungus (b) modelled using Kang et al. (2011) **A** without one-way transfer between chambers, and **B** with one-way transfer D = 0.1 between two chambers, with identical initial biomass conditions for the ants (B_a = 0.05) and symbiotic fungus (B_a = 0.3), and independent population dynamics within each chamber prior to one-way transfer.

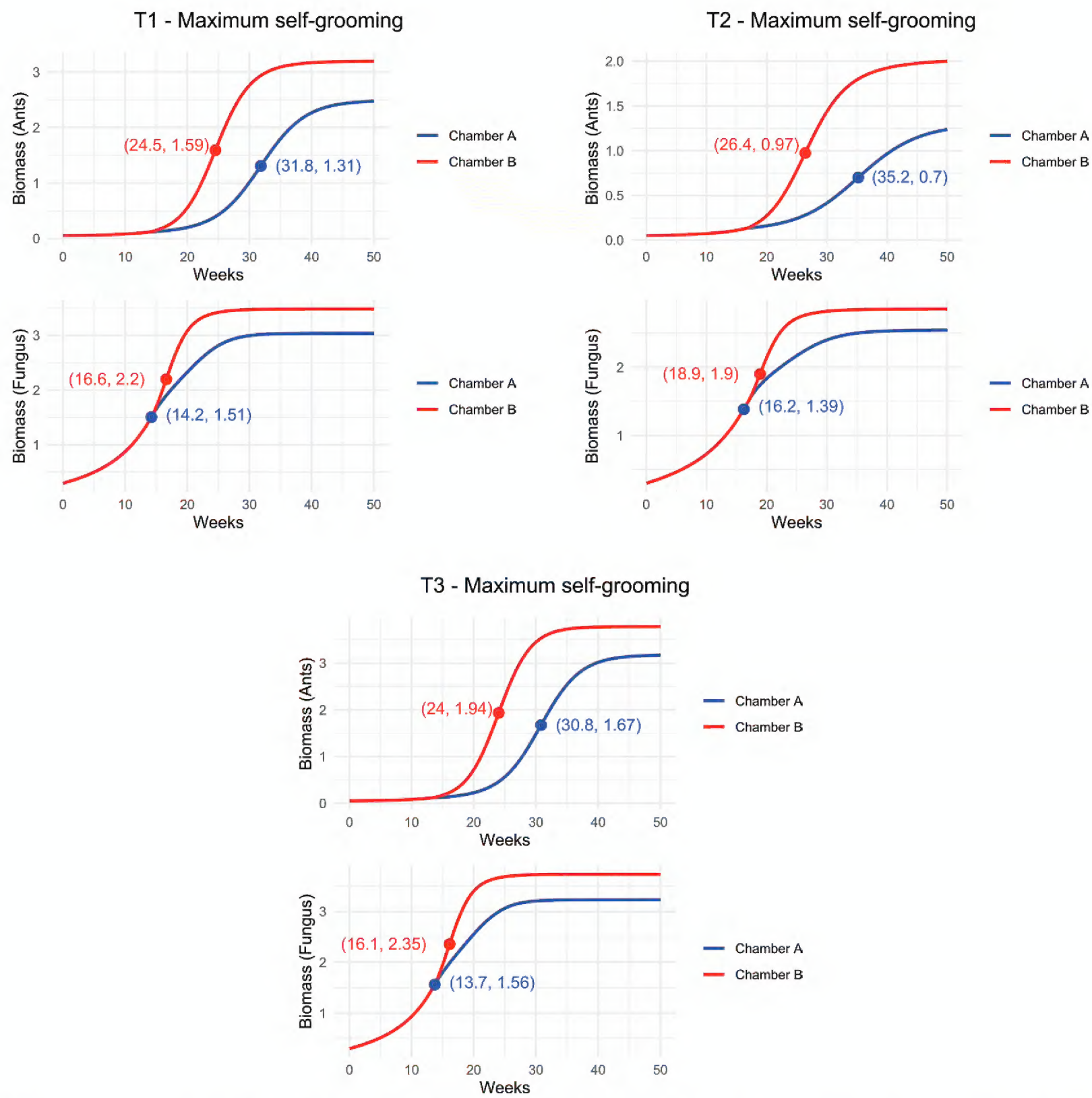

Figure 2. Maximum self-grooming effects on biomasses of leaf-cutting ants and symbiotic fungus with inflection points. Biomass growth curves with identified inflection points (solid blue circles) for chamber A and (solid red circles) for chamber B across three treatments: T1 (control), T2 (*Metarhizium anisopliae*), and T3 (*Escovopsis phialicopiosa*). *D* represents the one-way transfer of biomass from chamber A to chamber B ($D = 0.1$). The simulation uses maximum self-grooming durations measured in seconds: T1 (168 s), T2 (114 s), and T3 (185 s).

variations in their inflection values (Figs 6, 7). Excluding grooming protection (Fig. 8) led to a decrease in the biomasses of both the ants and the fungus.

The bifurcation diagram for worker allocation (q) compares minimum, mean, and maximum self-grooming times (Fig. 9). The general shape of all curves trends downwards, indicating that the proportion of workers cultivating the symbiotic fungus (q) approaches 0 when low biomass is produced, due to a low number of workers growing the fungus, and approaches 1 when a high number of workers excessively prioritise fungal growth, neglecting other tasks essential to the colony. A comparison of control (T1) and pathogenic fungi treatments (T2, T3) suggested that ant and symbiotic fungal biomasses were linked to peak self-grooming decreases under *M. anisopliae* (T2) but not under *E. phialicopiosa* (T3). Downward curves persist in Fig. 10; however, the ant and fungus biomasses were markedly reduced with *M. anisopliae* in both chambers *A* and *B*. The mean and minimum self-grooming times showed the same

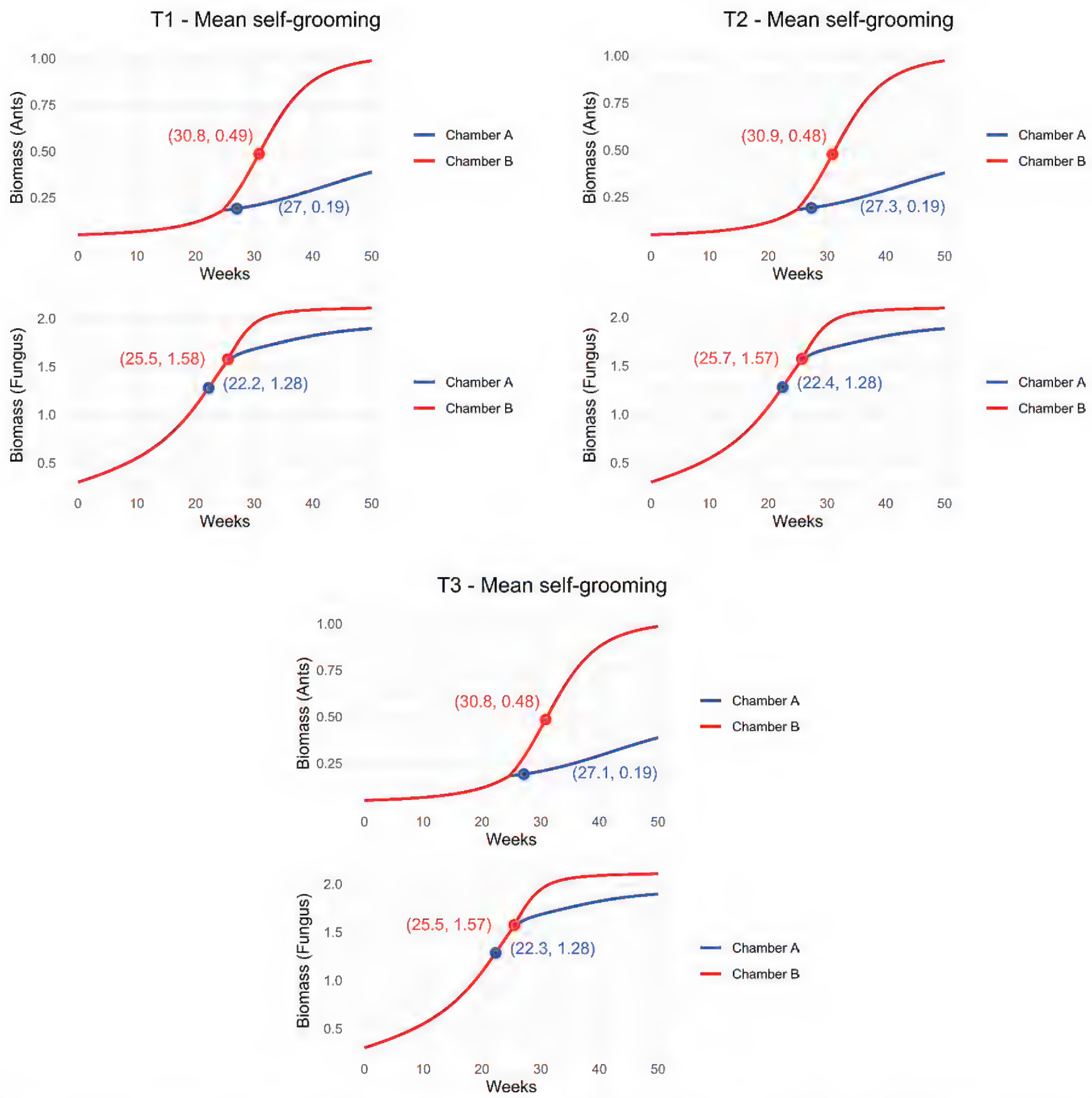

Figure 3. Mean self-grooming effects on biomasses of leaf-cutting ants and symbiotic fungus with inflection points. Biomass growth curves with identified inflection points (solid blue circles) for chamber A and (solid red circles) for chamber B across three treatments: T1 (control), T2 (*Metarhizium anisopliae*), and T3 (*Escovopsis phialicopiosa*). *D* represents the one-way transfer of biomass from chamber A to chamber B (D = 0.1). The model uses mean self-grooming durations measured in seconds: T1 (14.4 s), T2 (12 s), and T3 (14 s).

pattern observed for maximum self-grooming, although they differed quantitatively. Ant biomass associated with self-grooming was significantly higher than the biomass associated with allogrooming; however, symbiotic fungal biomass did not follow this pattern (see Figs 9, 10).

A general decrease in the biomasses of ants and fungus in response to increasing mortality values occurred for both ant mortality (d_a) and fungus mortality (d_f), considering the minimum, mean, and maximum values of s_t and a_t (Figs 11–14). Although the two mortality patterns were generally similar, important differences appeared when analysing d_a and d_f separately, from a grooming perspective.

Ant biomass in chamber *A* decreased non-linearly under self-grooming in all three treatments, with quantitative differences among the three (Fig. 11). A

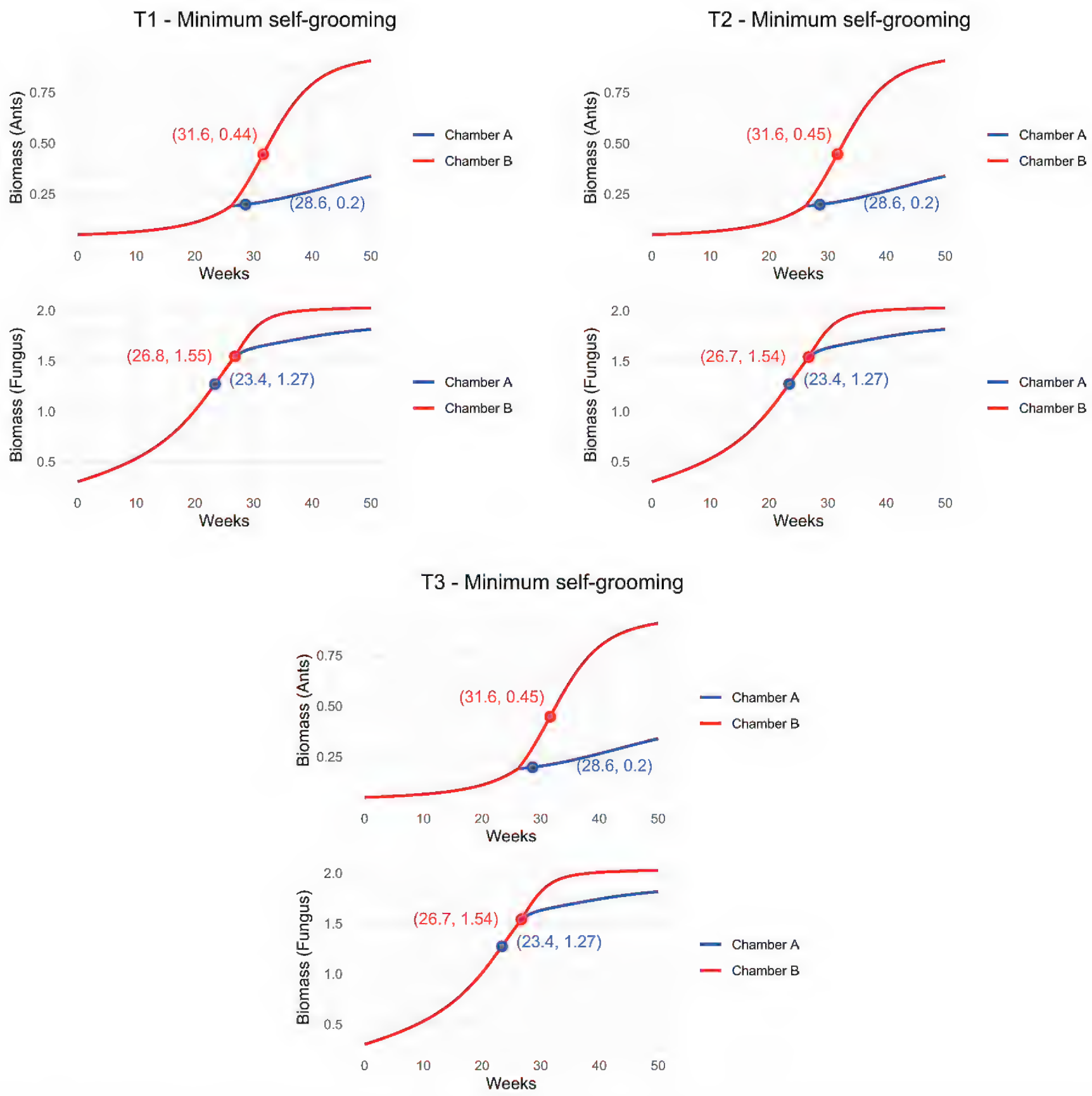

Figure 4. Minimum self-grooming effects on biomasses of leaf-cutting ants and symbiotic fungus with inflection points. Biomass growth curves with identified inflection points (solid blue circles) for chamber A and (solid red circles) for chamber B across three treatments: T1 (control), T2 (*Metarhizium anisopliae*), and T3 (*Escovopsis phialicopiosa*). *D* represents the one-way transfer of biomass from chamber A to chamber B (D = 0.1). The model uses minimum self-grooming durations measured in seconds: T1 (0.3 s), T2 (0.53 s), and T3 (0.73 s).

pronounced decrease in symbiotic fungus biomass as a function of d_a occurred only above d_a = 0.5, indicating a delayed impact on fungus biomass. The minimum and mean self-grooming levels impacted both the ants and the fungus similarly. Maximum self-grooming provided slightly greater protection for the ant biomass compared to the other grooming levels but provided significantly more protection for the fungus than for the ants (Fig. 11). Results for allogrooming were similar to those for self-grooming, except in T2, where ant and fungal biomass curves overlapped (Fig. 12). Notably, the ant biomass was lower in the allogrooming treatment T2 (Fig. 12) compared to T2 in the self-grooming treatment (Fig. 11). The data in Fig. 13 indicate that there was no significant difference in the reduction of ant and fungal biomasses among the three treatments. The same overlap observed in Fig. 12 was also noted in allogrooming for T2 (Fig. 14).

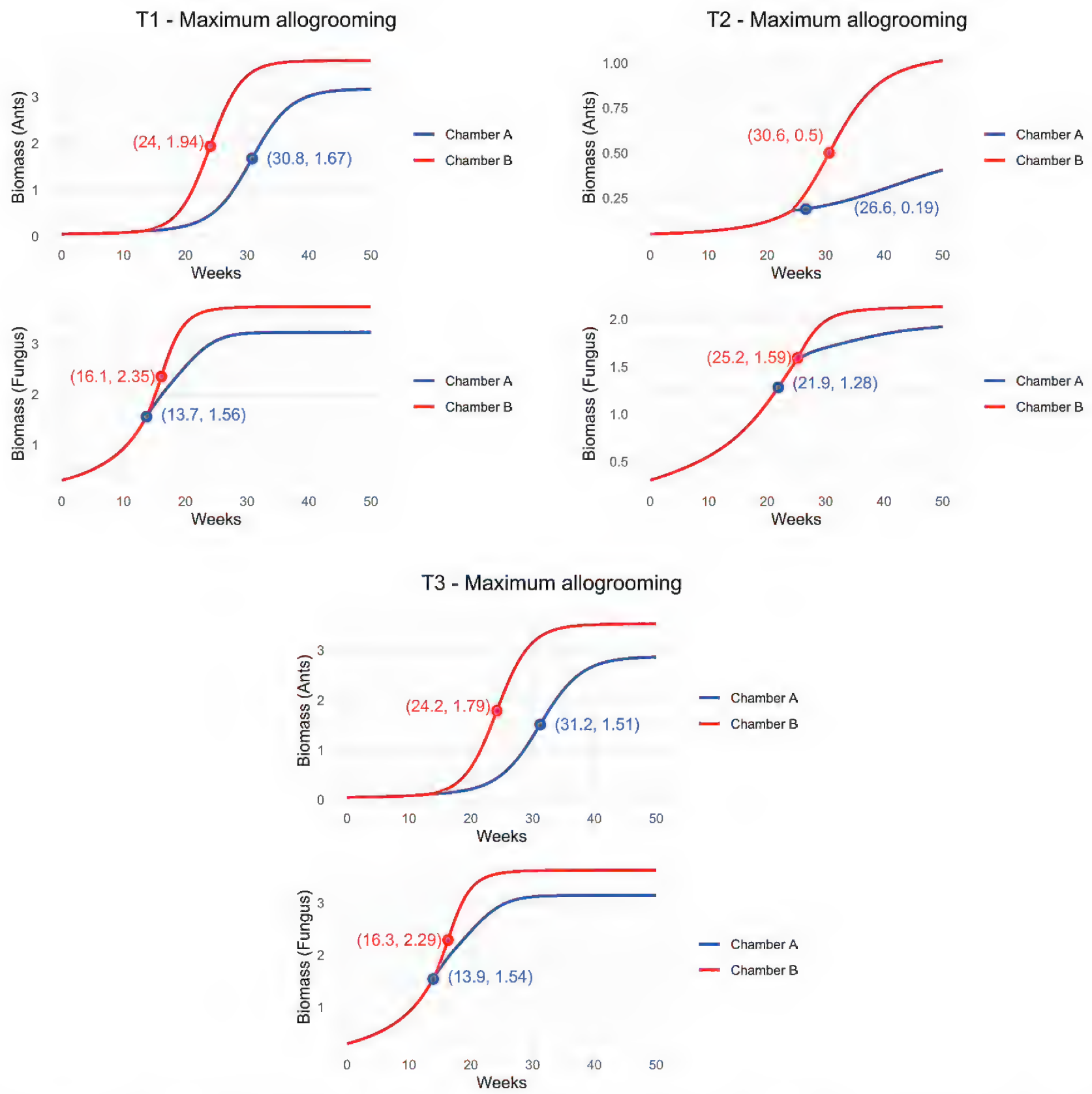

Figure 5. Maximum allogrooming effects on biomasses of leaf-cutting ants and symbiotic fungus with inflection points. Biomass growth curves with identified inflection points (solid blue circles) for chamber A and (solid red circles) for chamber B across three treatments: T1 (control), T2 (*Metarhizium anisopliae*), and T3 (*Escovopsis phialicopiosa*). *D* represents the one-way transfer of biomass from chamber A to chamber B (*D* = 0.1). The model uses maximum allogrooming durations measured in seconds: T1 (1701 s), T2 (169 s), and T3 (1639 s).

Discussion

The obligate symbiosis between leafcutter ants and basidiomycete fungi is based on a bidirectional dependency: the ants provide fresh plant substrate and protection to the symbiotic fungus, while the fungus serves as the exclusive nutritional source for the colony (Weber 1966; Mehdiabadi and Schultz 2010; Hölldobler and Wilson 2011). The stability of this mutualism is maintained through complex behavioural mechanisms, including dynamic worker allocation (Constantino et al. 2021) and individual (Currie and Stuart 2001) and collective hygiene strategies (Cremer et al. 2007). The introduction of pathogens disrupts this homeostasis, revealing critical resilience thresholds.

The simulations performed here were based on the model of Kang et al. (2011) but expanded to include the one-way chamber transfer parameter,

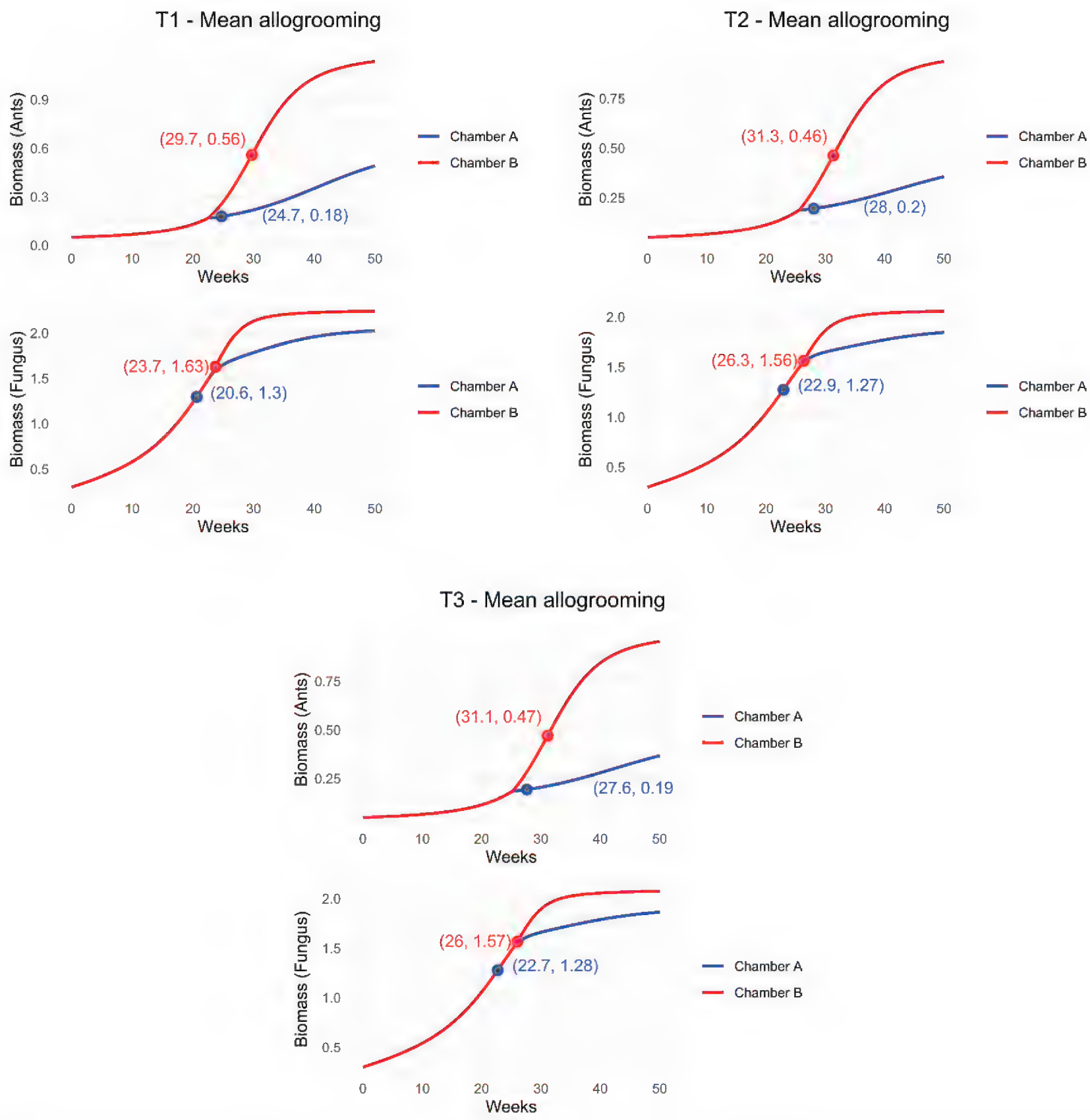

Figure 6. Mean allogrooming effects on biomasses of leaf-cutting ants and symbiotic fungus with inflection points. Biomass growth curves with identified inflection points (solid blue circles) for chamber A and (solid red circles) for chamber B across three treatments: T1 (control), T2 (*Metarhizium anisopliae*), and T3 (*Escovopsis phialicopiosa*). *D* represents the one-way transfer of biomass from chamber A to chamber B ($D = 0.1$). The model uses mean allogrooming durations measured in seconds: T1 (330 s), T2 (54 s), and T3 (82 s).

demonstrating how variations in ant mortality (d_a) and fungal mortality (d_f) drastically alter the system biomass (Fig. 1B). The one-way transfer parameter (D) quantified biomass exchange of ants and the symbiotic fungus between nest chambers, reflecting ecological differences in nest architecture between *Atta* species with multi-chambered nests (Moreira et al. 2010) and *Acromyrmex* species, which typically have single-chambered nests (Forti et al. 2006).

For $D > 0$, one-way transfer redistributes resources (Fig. 1B), reducing biomass in the source chamber (A) whilst increasing it in chamber (B). This dynamic is particularly critical for *Atta*, where multi-chambered nests mitigate local stresses, such as pathogen outbreaks, by reallocating workers and fungal biomass. This is evidenced by delayed inflection points observed under $D = 0.1$ (Fig. 2). Future studies extending this model to interconnected multi-chamber networks could explore one-way transfer patterns, resilience, and epidemiological

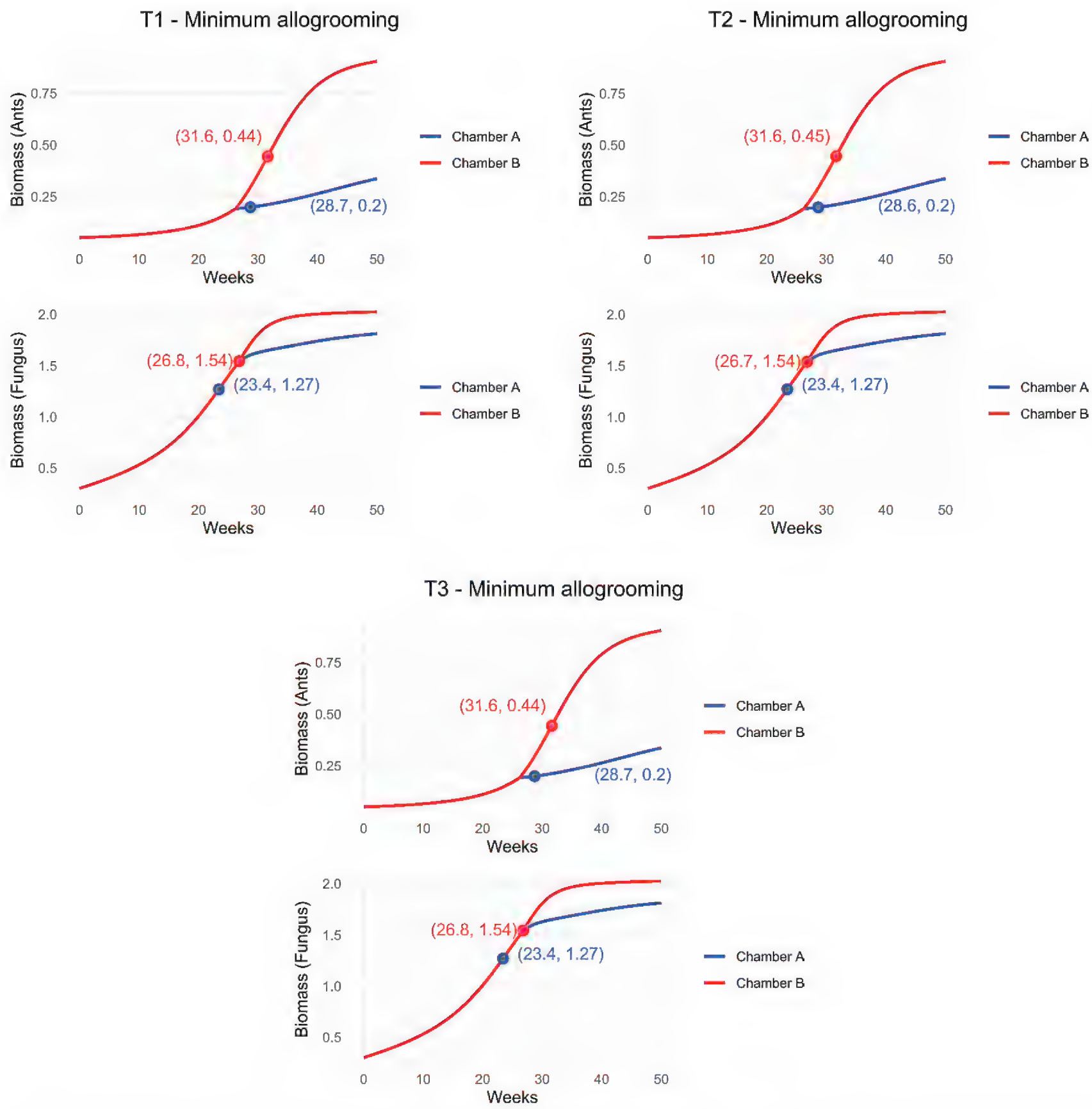

Figure 7. Minimum allogrooming effects on biomasses of leaf-cutting ants and symbiotic fungus with inflection points. Biomass growth curves with identified inflection points (solid blue circles) for chamber A and (solid red circles) for chamber B across three treatments: T1 (control), T2 (*Metarhizium anisopliae*), and T3 (*Escovopsis phialicopiosa*). *D* represents the one-way transfer of biomass from chamber A to chamber B (*D* = 0.1). The model uses minimum allogrooming durations measured in seconds: T1 (1.5 s), T2 (3.87 s), and T3 (1.23 s).

processes within nest topology, revealing how chamber complexity influences ecological stability.

The sigmoidal patterns observed in the biomasses align with classical models of obligate mutualism (Vandermeer and Boucher 1978). Introducing a second fungal chamber reveals critical specificities, with the reduced biomass in chamber *A* suggesting an energetic cost in resource reallocation. The maintenance of population stabilisation, even under one-way transfer, underscores the behavioural plasticity of ants in worker allocation (Gordon 1996), a key mechanism for mutualistic resilience. The experimental introduction of pathogens, such as *M. anisopliae* (T2) and *E. phialicopiosa* (T3), into the colonies had distinct results. *Metarhizium anisopliae* significantly reduced ant and symbiotic fungus biomasses, although the most marked differences among treatments arose from maximum self-grooming

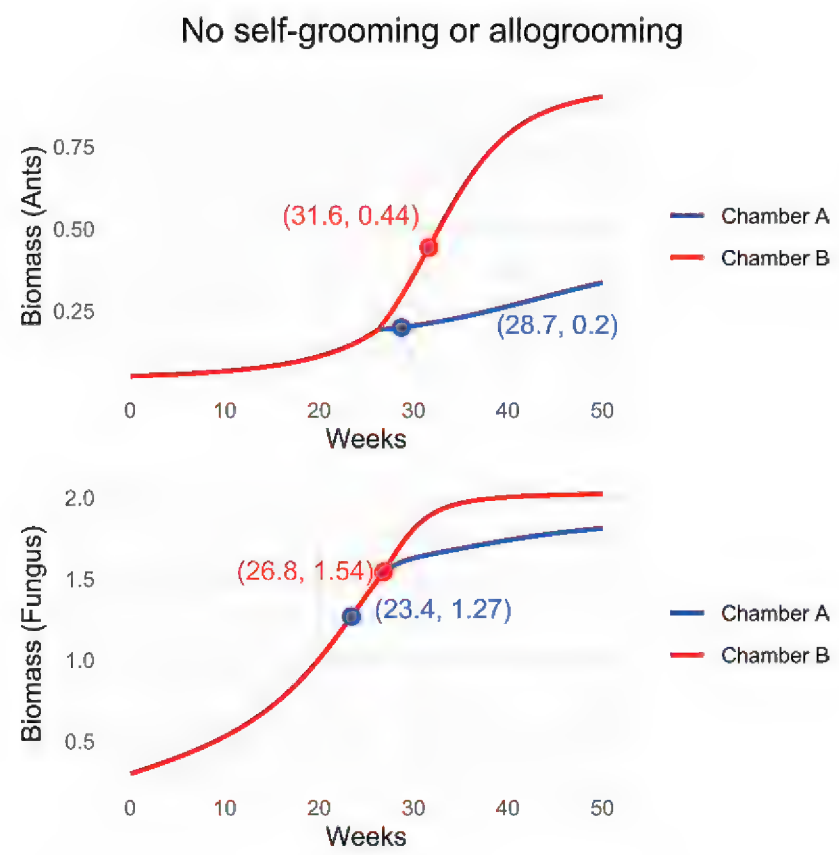

Figure 8. Effects of the absence of self-grooming and allogrooming on biomasses of leaf-cutting ants and symbiotic fungus with inflection points. Biomass growth curves with identified inflection points (solid blue circles) for chamber A and (solid red circles) for chamber B under the condition where no grooming behaviour occurs. *D* represents the one-way transfer of biomass from chamber A to chamber B (D = 0.1).

and allogrooming levels. Even at maximum levels of self-grooming and allogrooming, *Metarhizium anisopliae* infection was not eliminated.

The impact of *M. anisopliae* was evident even under moderate symbiotic fungus mortality ($d_f \approx 0.4–0.5$, Figs 13, 14), directly compromising workers and the maintenance of the fungal garden. In contrast, *E. phialicopiosa* exhibited effects comparable to the control in most scenarios, with pronounced impacts only under high fungal mortality, at least examining d_f influenced by allogrooming ($d_f > 0.5$, Fig. 14). In treatment T2 (*Metarhizium anisopliae*), ant and fungal mortality curves overlapped among all allogrooming levels (max, mean, min; Figs 12, 14), indicating that social grooming fails to decouple the pathogen's impact on both mutualistic partners. Although *Escovopsis* spp. are primarily pathogenic to the ants' cultivated symbiotic fungus (typically *Leucoagaricus* or *Leucocoprinus basidiomycetes*) rather than to the ants themselves, their degradation of fungal gardens disrupts the colony's food supply, threatening its survival. These simulations align with literature indicating that *E. phialicopiosa* acts opportunistically, exploiting the symbiotic fungus' vulnerability under high mortality conditions (Jiménez-Gómez et al. 2021; Mendonça et al. 2021). Additionally, the limited impact of *E. phialicopiosa* may stem from the lower virulence of the strain (Montoya et al. 2023), which fails to trigger strong physiological or behavioural responses.

In contrast, the pronounced effects of *M. anisopliae* on both mutualistic components suggest a disruption to intra-colony labour allocation. As *M. anisopliae* infects insects via conidial adhesion to the cuticle and subsequent tegument penetration (Shah and Pell 2003), self-grooming alone may be insufficient to prevent infection despite reducing spore quantity and viability (Hughes et al. 2002). Workers may thus prioritise alternative defences, such as increasing the frequency of self-grooming over its duration, cleaning the fungal garden (Goes et al. 2022), or activating physiological defences integrated with behavioural responses (Fernández-Marín et al. 2006), which were not quantified in this study.

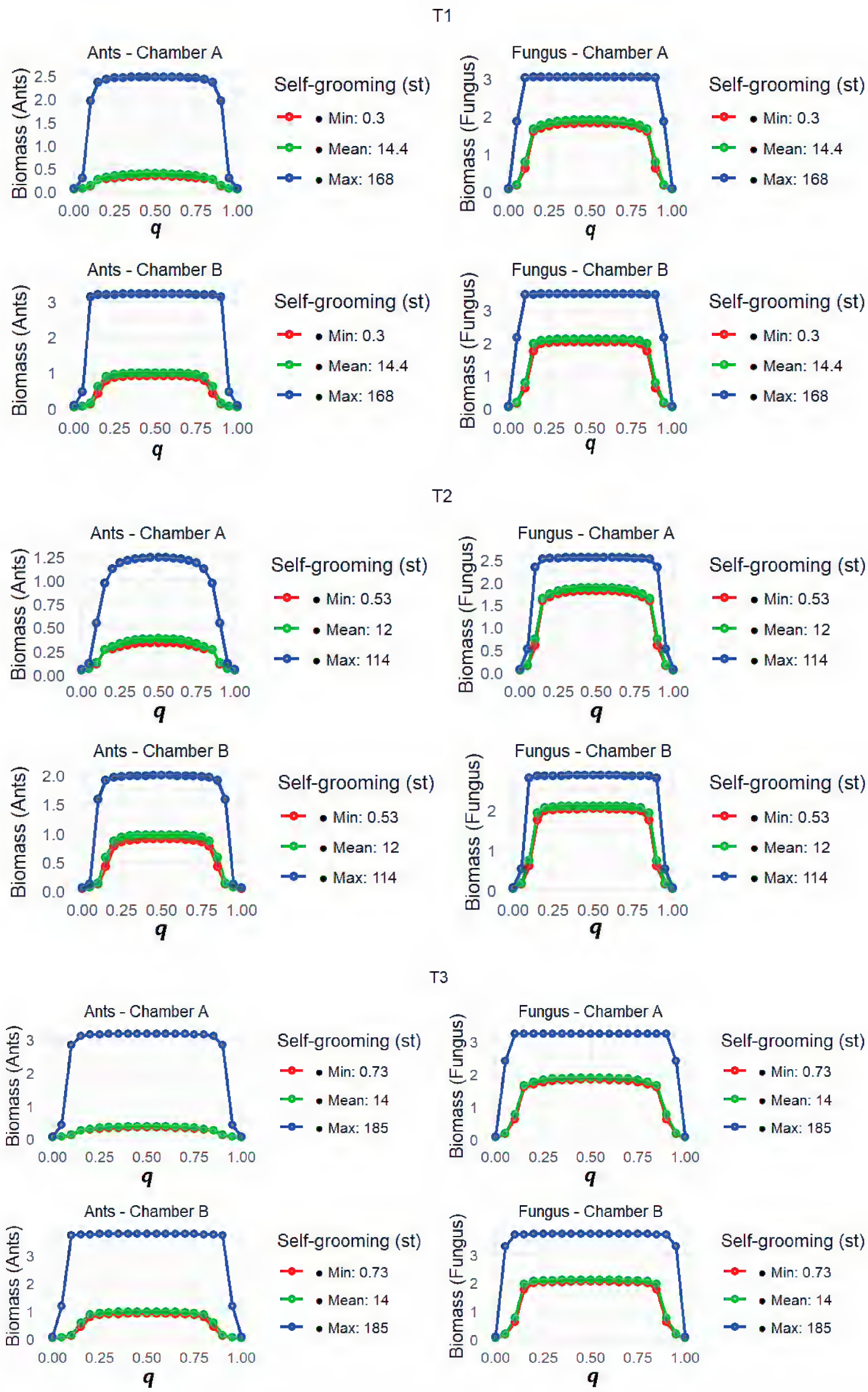

Figure 9. Bifurcation diagrams for *q* (the proportion of workers cultivating the symbiotic fungus) in T1 (control), T2 (*Metarhizium anisopliae*), and T3 (*Escovopsis phialicopiosa*), considering minimum (red), mean (green), and maximum (blue) time spent on self-grooming.

Infection may also impair worker activity, as evidenced by delayed ant and fungal biomass stabilisation under maximum self-grooming. Studies have shown that *M. anisopliae* can overwhelm workers and disrupt essential activities such

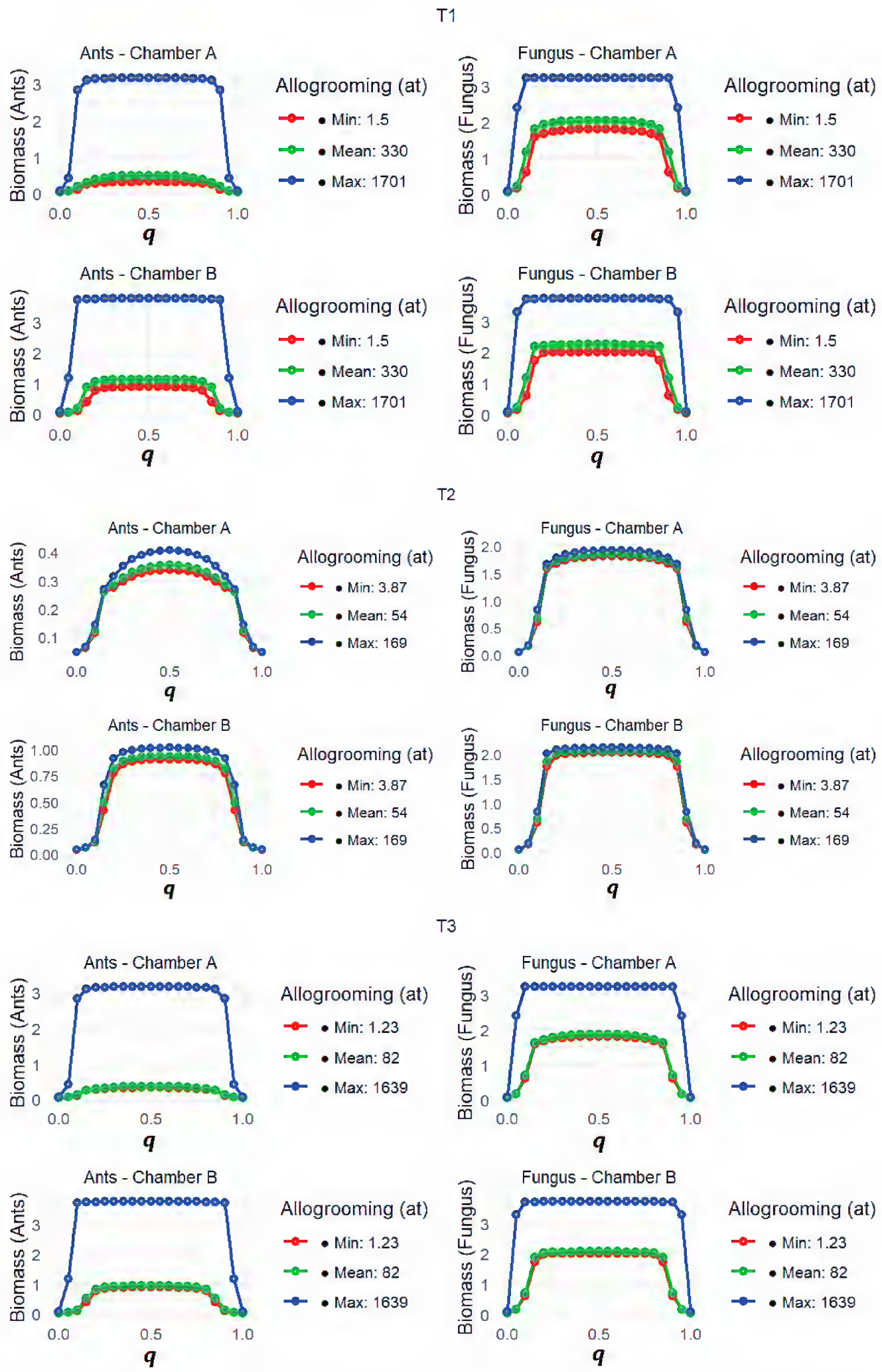

Figure 10. Bifurcation diagrams for q (the proportion of workers cultivating the symbiotic fungus) in T1 (control), T2 (*Metarhizium anisopliae*), and T3 (*Escovopsis phialicopiosa*), considering minimum (red), mean (green), and maximum (blue) time spent on allogrooming.

as foraging (Jaccoud et al. 1999), suggesting that infection-induced destabilisation of social homeostasis is a key factor in colony decline. These results align with empirical studies reporting similar responses in leaf-cutting ants challenged with *Beauveria bassiana* and *Trichoderma harzianum* (Mota-Filho et al. 2021).

Whilst the factors regulating grooming intensity in leaf-cutting ants are not fully understood, they are generally considered multifaceted (Goes et al. 2022). Several hypotheses could be proposed to explain this variation, including behavioural effort linked to pathogen prevalence and infection risks, duration of exposure to

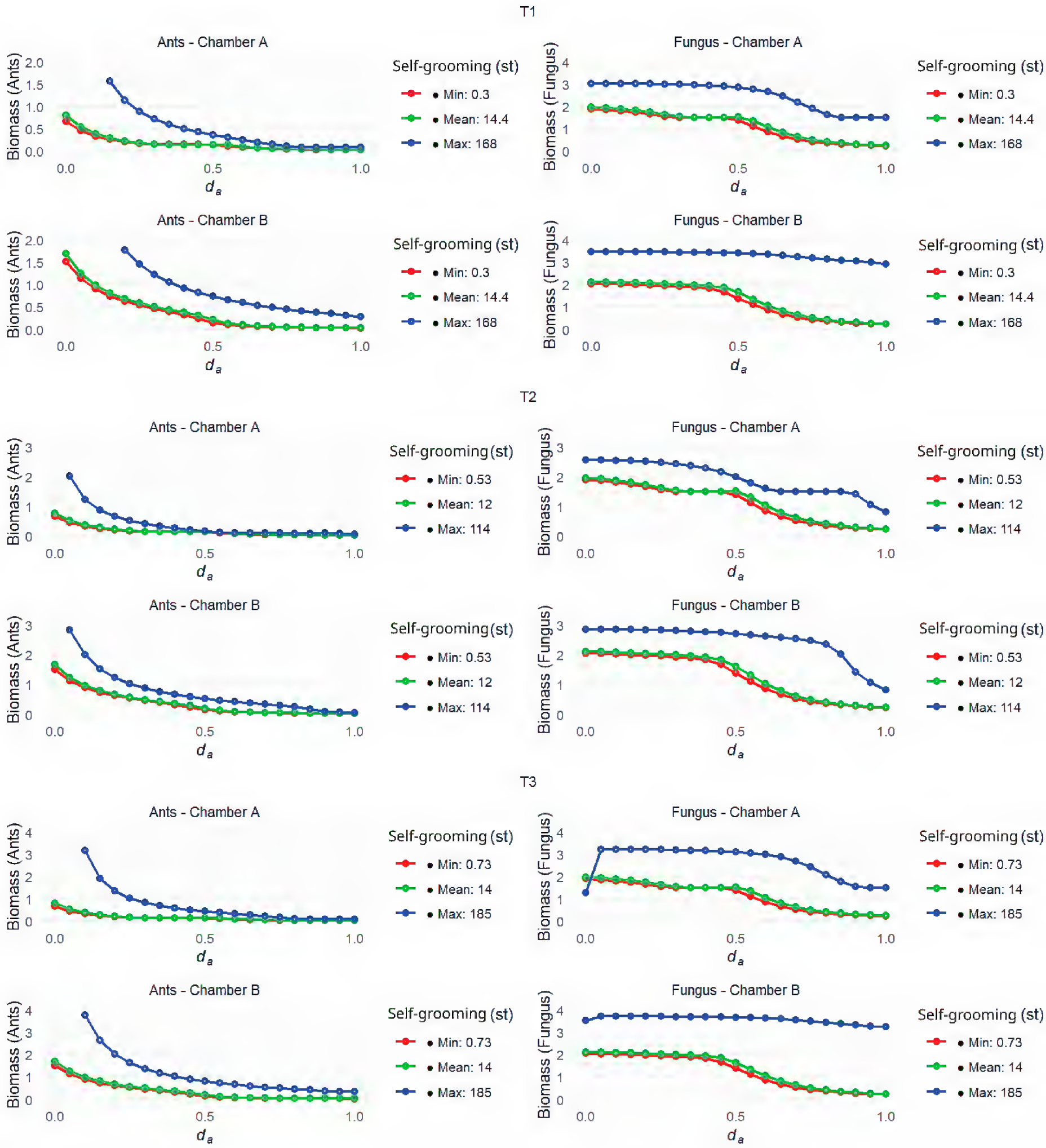

Figure 11. Bifurcation diagrams for d_a (mortality rate of ants) in T1 (control), T2 (*Metarhizium anisopliae*), and T3 (*Escovopsis phialicopiosa*), considering minimum (red), mean (green), and maximum (blue) time spent on self-grooming.

pathogens or contaminants, age polyethism, colony-level genetic diversity, queen pheromone-mediated modulation, and environmental stressors such as temperature and humidity. However, it remains to be answered whether and how much such factors contribute to regulating the grooming intensity in leaf-cutting ants.

Our simulations indicate that hygiene behaviours differentially affect the system biomass. Self-grooming proved more effective against *M. anisopliae* than allogrooming, reflecting the prioritisation of individual defences under high pathogen pressure (Figs 2 T2, 5 T2). This aligns with observations that ants modulate allogrooming in the presence of virulent pathogens to minimise horizontal spore transmission (Theis et al. 2015; Konrad et al. 2018). The lack

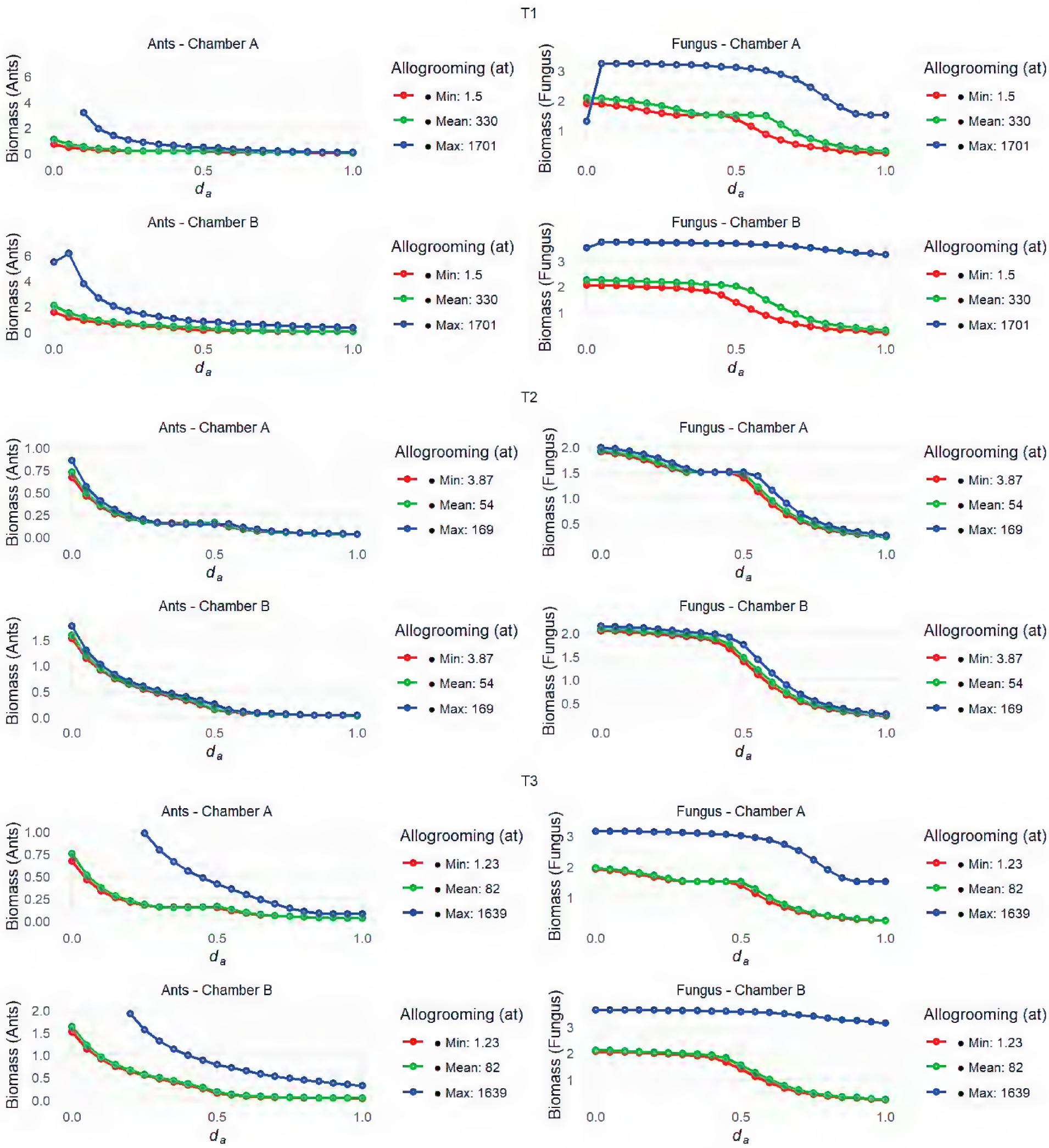

Figure 12. Bifurcation diagrams for d_a (mortality rate of ants) in T1 (control), T2 (*Metarhizium anisopliae*), and T3 (*Escovopsis phialicopiosa*), considering minimum (red), mean (green), and maximum (blue) time spent on allogrooming.

of impact of allogrooming on *E. phialicopiosa* likely stems from the pathogen's lower virulence (Fig. 5 T1, T3). However, under high fungal mortality ($d_f > 0.5$), allogrooming may accelerate system collapse due to secondary spore transmission and opportunistic behaviour (Jiménez-Gómez et al. 2021).

The relationship between ant mortality (d_a) and population collapse revealed a critical threshold ($d_a \approx 0.5$) beyond which the mutualism loses resilience. For *M. anisopliae*, high worker mortality directly impaired fungal care, accelerating the decline in fungal biomass ($d_f > 0.4$) even under maximum self-grooming, demonstrating a critical threshold for mutualistic collapse (Fig. 13). The fungal decline

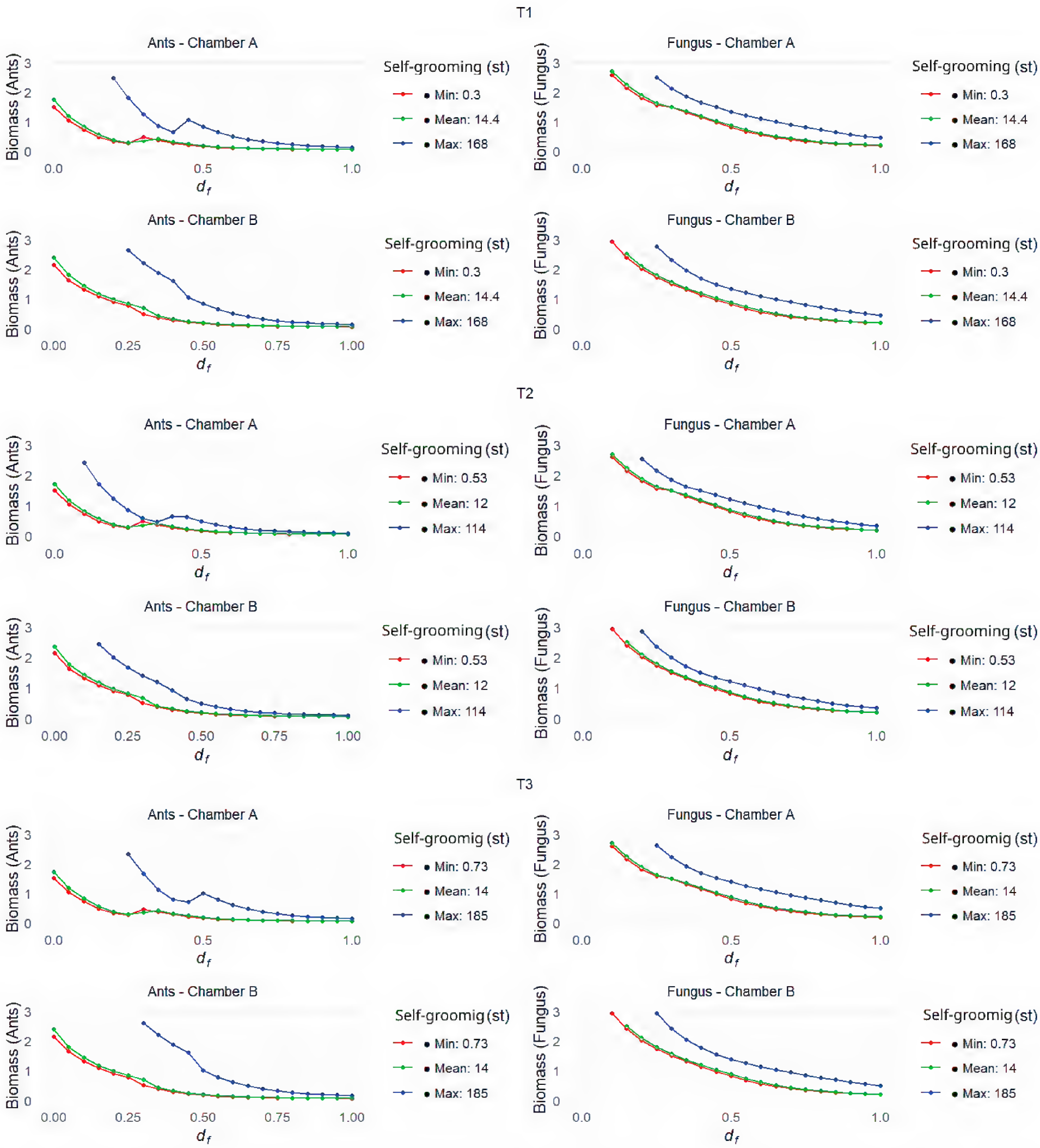

Figure 13. Bifurcation diagrams for d_a (mortality rate of fungus) in T1 (control), T2 (*Metarhizium anisopliae*), and T3 (*Escovopsis phialicopiosa*), considering minimum (red), mean (green), and maximum (blue) time spent on self-grooming.

is not due solely to reduced care but also to the collapse of symbiotic interactions (Mueller et al. 2005; Dejean et al. 2023). In contrast, *E. phialicopiosa* (T3) exhibited a threshold-dependent response to fungal mortality, with pronounced effects only when the symbiont's baseline mortality exceeded df > 0.5. Under self-grooming, T3 and T1 were indistinguishable at low-to-moderate mortality levels (Fig. 13).

Bifurcation diagrams revealed critical system transitions (Figs 9, 10). Under low biomass ($q \rightarrow 0$ or $q \rightarrow 1$), colonies had to choose between prioritising fungal care ($q \rightarrow 1$) or abandoning it ($q \rightarrow 0$). Intermediate values (q = 0.4

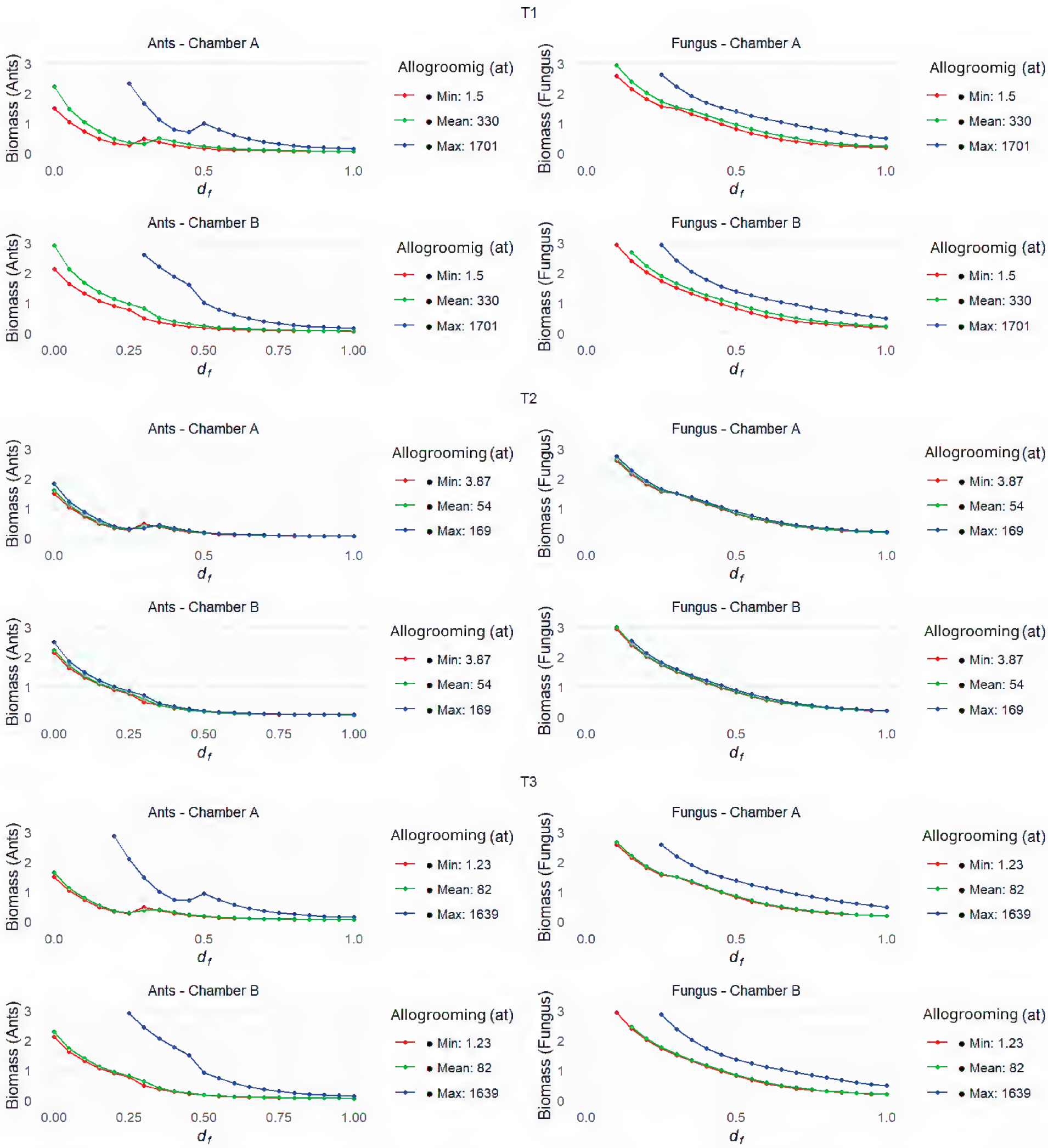

Figure 14. Bifurcation diagrams for d_f (total mortality of fungus) in T1 (control), T2 (*Metarhizium anisopliae*), and T3 (*Escovopsis phialicopiosa*), considering minimum (red), mean (green), and maximum (blue) time spent on allogrooming.

to 0.6) maximised stability by balancing essential functions. The parameter q represents the proportion of workers allocated to fungus cultivation. Therefore, when biomass is low and q approaches zero, few workers are available to cultivate the symbiotic fungus, resulting in reduced biomass levels. Conversely, when q approaches 1, workers are fully dedicated to fungus cultivation, focusing exclusively on this task. Consequently, other tasks may be neglected, to the detriment of overall colony function, in favour of maximising fungus growth.

Delayed inflection points in biomass curves influenced by *M. anisopliae* likely arise from high pathogen intensity (k_a), which increases worker mortality (d_a),

impairs fungal care, and initially prioritises self-grooming over allogrooming (Figs 2 T2, 5 T2). This creates a lag between pathogen exposure and mutualistic collapse. The critical threshold ($d_a \approx 0.5$) marks the point at which worker mortality exceeds the colony's replacement capacity (r_a), accelerating fungal decline (Fig. 11). Reduced fungal care ($q < 0.25$) explains the loss of resilience beyond this threshold (Figs 10, 11).

These results provide critical insights into pathogen transmission dynamics within leaf-cutting colonies and the factors shaping the resilience of ant-fungus mutualism. Beyond ecological understanding, these findings could inform practical strategies for managing *Atta* spp., which are significant agricultural pests.

Conclusion

The results highlight the critical role of multi-chamber nest architecture and hygienic practices in mitigating pathogen impacts. *Metarhizium anisopliae* was identified as a more severe threat to colony stability compared to *Escovopsis phialicopiosa*, significantly reducing both ant and fungal biomasses. Self-grooming proved more effective than allogrooming in countering *M. anisopliae*, suggesting that individual defences are prioritised under high pathogen pressure. The study also revealed critical thresholds for ant and fungal mortality, beyond which the mutualistic system collapses. Worker allocation to fungus cultivation emerged as a key factor in maintaining stability, with intermediate proportions optimising resilience. These findings enhanced our understanding of the ecological and behavioural strategies that support the resilience of ant-fungus mutualisms and provided insights for developing targeted biological control strategies against leaf-cutting ants.

Acknowledgements

We express our sincere gratitude to the funding body CNPq and grant #2023/08286-2, São Paulo Research Foundation (FAPESP), for their support and contribution to this research. We also thank Dr Ricardo Toshio Fujihara for kindly providing the ant colonies used in this study. We are grateful to Dr Janet Reid for proofreading this article in English. We are also grateful to two anonymous reviewers who contributed significantly to improving the manuscript.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This work was supported by CNPq - PhD 202308286-2.

Author contributions

Formal analysis: LSC. Methodology: JVMFT. Writing – original draft: IB. Writing – review and editing: WACG.

Author ORCIDs

Isabella Bueno https://orcid.org/0000-0002-1241-066X
João Vitor Mendes Ferraz de Toledo https://orcid.org/0009-0003-5525-5158
Lucas Santos Canuto https://orcid.org/0009-0000-6854-4774
Wesley Augusto Conde Godoy https://orcid.org/0000-0002-2619-7476

Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

References

Bertorelli MV, Montilla J, Hernández J (2006) Efecto de la defoliación por hormigas cortadoras de hojas (Formicidae: Attini) sobre el rendimiento de la yuca (Manihot esculenta CRANTZ). Revista de la Facultad de Agronomía 23(3): 310–318.

Bucher E (1982) Chaco and Caatinga-South American arid savannas, woodlands and-tickets. In: Huntley B, Walker B (Eds) Ecology of tropical savannas. Springer-Verlag, Berlin, 48–79. https://doi.org/10.1007/978-3-642-68786-0_4

Constantino SM, Schlüter M, Weber EU, Wijermans N (2021) Cognition and behavior in context: A framework and theories to explain natural resource use decisions in social-ecological systems. Sustainability Science 16(5): 1651–1671. https://doi.org/10.1007/s11625-021-00989-w

Costa AN, Vasconcelos HL, Vieira-Neto EHN, Bruna EM (2008) Do herbivores exert top-down effects in Neotropical savannas? Journal of Vegetation Science 19(6): 849–854. https://doi.org/10.3170/2008-8-18461

Cremer S, Armitage SAO, Schmid-Hempel P (2007) Social immunity. Current Biology 17(16): R693–R702. https://doi.org/10.1016/j.cub.2007.06.008

Currie CR, Stuart AE (2001) Weeding and grooming of pathogens in agriculture by ants. Proceedings. Biological Sciences 268: 1033–1039. https://doi.org/10.1098/rspb.2001.1605

Currie CR, Mueller UG, Malloch D (1999) The agricultural pathology of ant fungus gardens. Proceedings of the National Academy of Sciences of the United States of America 96(14): 7998–8002. https://doi.org/10.1073/pnas.96.14.7998

Dejean A, Azémar F, Naskrecki P, Tindo M, Rossi V, Faucher C (2023) Mutualistic interactions between ants and fungi: A review. Ecology and Evolution 13: e10386. https://doi.org/10.1002/ece3.10386

Delabie JCH, Alves HSR, Reuss-Strenzel GM, Carmo AD, Nascimento ID (2011) Distribuição das formigas cortadeiras *Acromyrmex* e *Atta* no Novo Mundo. In: Della-Lucia (Ed.) Formigas-cortadeiras: da bioecologia ao manejo. UFV, 80–101.

Farji-Brener AG, Tadey M (2012) Trash to treasure: Leaf-cutting ants repair nest-mound damage by recycling refuse dump materials. Behavioral Ecology 23(10): 1195–1202. https://doi.org/10.1093/beheco/ars101

Fernández-Marín H, Zimmerman JK, Rehner AS, Wcislo WT (2006) Active use of the metapleural glands by ants in controlling fungal infection. Proceedings of the Royal Society 273: 1689–1695. https://doi.org/10.1098/rspb.2006.3492

Forti LC, Andrade ML, Andrade APP, Lopes JFS, Ramos VN (2006) Bionomics and identification of *Acromyrmex* (Hymenoptera: Formicidae) through an illustrated key. Sociobiology 48: 135–153. https://www.academia.edu/27198755/Bionomics_and_identification_of_

Forti LC, Rando JS, Camargo RS, Moreira AA, Castellani MA, Leite SA, Sousa KKA, Caldato N (2020) Occurrence of leaf-cutting and grass-cutting ants of the genus *Atta*

(Hymenoptera: Formicidae) in geographic regions of Brazil. Sociobiology 67(4): 514–525. https://doi.org/10.13102/sociobiology.v67i4.5741

Fowler N (1986) The role of competition in plant communities in arid and semi-arid regions. Annual Review of Ecology and Systematics 17: 89–110. https://doi.org/10.1146/annurev.es.17.110186.000513

Goes AC, Kooij PW, Culot L, Bueno OC, Rodrigues A (2022) Distinct and enhanced hygienic responses of a leaf-cutting ant toward repeated fungi exposures. Ecology and Evolution 12: e9112. https://doi.org/10.1002/ece3.9112

Gordon DM (1996) The organization of work in social insect colonies. Nature 380: 121–124. https://doi.org/10.1038/380121a0

Hart AG, Ratnieks FLW (2002) Waste management in the leaf-cutting ant *Atta colombica*. Behavioral Ecology 13(2): 224–231. https://doi.org/10.1093/beheco/13.2.224

Holland JN, DeAngelis DL (2010) A consumer–resource approach to the density-dependent population dynamics of mutualism. Ecology 91: 1286–1295. https://doi.org/10.1890/09-1163.1

Hölldobler B, Wilson EO (2011) The leafcutter ants. W. W. Norton & Co., New York, 1–192.

Hughes WOH, Eilenberg J, Boomsma JJ (2002) Trade-offs in group living: transmission and disease resistance in leaf-cutting ants. Proceedings of the Royal Society of London B 269: 1811–1819. https://doi/org/10.1098/rspb.2002.2113

Jaccoud DB, Hughes WHO, Jackson CW (1999) The epizootiology of a *Metarhizium* infection in mini-nests of the leaf-cutting ant *Atta sexdens rubropilosa*. Entomologia Experimentalis et Applicata 93: 51–61. https://doi.org/10.1046/j.1570-7458.1999.00561.x

Jiménez-Gómez I, Barcoto MO, Montoya QV, Goes AC, Monteiro LSVE, Bueno OC, Rodrigues A (2021) Host Susceptibility Modulates *Escovopsis* Pathogenic Potential in the Fungiculture of Higher Attine Ants. Frontiers in Microbiology 12: 673444. https://doi.org/10.3389/fmicb.2021.673444

Kang Y, Clark R, Makiyama M, Fewell J (2011) Mathematical modeling on obligate mutualism: Interactions between leaf-cutter ants and their fungus garden. Journal of Theoretical Biology 289: 116–127. https://doi.org/10.1016/j.jtbi.2011.08.027

Konrad M, Pull CD, Metzler S, Self K, Naderlinger E, Grasse AV, Cremer S (2018) Ants avoid superinfections by performing risk-adjusted sanitary care. Proceedings of the National Academy of Sciences of the United States of America 115: 2782–2787. https://doi.org/10.1073/pnas.1713501115

Lima EABF, Ferreira CP, Godoy WAC (2009) Ecological modeling and pest population management: A possible and necessary connection in a changing world. Neotropical Entomology 38(6): 699–707. https://doi.org/10.1590/S1519-566X2009000600001

Mariconi FAM (1970) As saúvas. São Paulo, Brazil. Editora Agronômica Ceres 1: 167.

Mehdiabadi NJ, Schultz TR (2010) Natural history and phylogeny of the fungus-farming ants (Hymenoptera: Formicidae: Myrmicinae: Attini). Myrmecological News 13: 37–55. https://api.semanticscholar.org/CorpusID:35563788

Mendonça DMF, Caixeta MCS, Martins GL, Moreira CC, Kloss TG, Elliot SL (2021) Low virulence of the fungi *Escovopsis* and *Escovopsioides* to a leaf-cutting ant-fungus symbiosis. Frontiers in Microbiology 12: 673445. https://doi.org/10.3389/fmicb.2021.673445

Montoya QV, Martiarena MJS, Rodrigues A (2023) Taxonomy and systematics of the fungus-growing ant associate *Escovopsis* (Hypocreaceae). Studies in Mycology 106: 349–397. https://doi.org/10.3114/sim.2023.106.06

Moreira DDO, Viana-Bailez AMM, Erthal Jr M, Bailez O, Carrera MP, Samuels RI (2010) Resource allocation among worker castes of the leaf-cutting ants *Acromyrmex sub-*

terraneus subterraneus through trophallaxis. Journal of Insect Physiology 56(11): 1665–1670. https://doi.org/10.1016/j.jinsphys.2010.06.018

Mota-Filho TMM, Stefanelli LEP, Camargo RS, Matos CAO, Forti LC (2021) Biological control in leaf-cutting ants, *Atta sexdens* (Hymenoptera: Formicidae), using pathogenic fungi. Revista Árvore 45: e4516. https://doi.org/10.1590/1806-908820210000016

Mueller UG, Gerardo NM, Aanen DK (2005) The evolution of agriculture in insects. Annual Review of Ecology, Evolution, and Systematics 36: 563–595. https://doi.org/10.1146/annurev.ecolsys.36.102003.152626

Real LA (1977) The kinetics of functional response. American Naturalist 11: 289–300. https://doi.org/10.1086/283161

Rissanen J, Helanterä H, Freitak D (2022) Pathogen prevalence modulates medication behavior in ant *Formica fusca*. Frontiers in Insect Science 27(2): 870971. https://doi.org/10.3389/finsc.2022.870971

Römer D, Roces F (2014) Nest enlargement in leaf-cutting ants: Relocated brood and fungus trigger the excavation of new chambers. PLoS ONE 9(5): e97872. https://doi.org/10.1371/journal.pone.0097872

Scherf AN, Corley JC, Gioia CD, Eskiviski ER, Carazzo C, Patzer HR, Dimarco RD (2022) Impact of a leaf-cutting ant (*Atta sexdens* L.) on a *Pinus taeda* plantation: A 6 year-long study. Journal of Applied Entomology 146(9): 1178–1184. https://doi.org/10.1111/jen.13047

Schmid-Hempel P (1998) Parasites in Social Insects. Princeton University Press, Princeton, 1–392. https://doi.org/10.1515/9780691206851

Schultz TR, Sosa-Calvo J, Kweskin MP, Lloyd MW, Dentinger B, Kooij PW, Vellinga EC, Rehner SA, Rodrigues A, Doyle VP, Montoya QV, Fernández-Marín H, Ješovnik A, Niskanen T, Liimatainen K, Leal-Dutra CA, Solomon SE, Gerardo NM, Currie CR, Bacci Jr M, Vasconcelos HL, Rabeling C, Faircloth BC, Doyle VP (2024) The coevolution of fungus-ant agriculture. Science 386(6717): 105–110. https://doi.org/10.1126/science.adn7179

Shah PA, Pell JK (2003) Entomopathogenic fungi as biological control agents. Applied Microbiology and Biotechnology 61: 413–423. https://doi.org/10.1007/s00253-003-1240-8

Soetaert K, Petzoldt T, Setzer RW (2010) Solving differential equations in r: Package desolve. Journal of Statistical Software 33(9): 1–25. https://doi.org/10.18637/jss.v033.i09

Theis FJ, Ugelvig LV, Marr C, Cremer S (2015) Opposing effects of allogrooming on disease transmission in ant societies. Philosophical Transactions of the Royal Society of London, Series B 370(1669): 20140108. https://doi.org/10.1098/rstb.2014.0108

Vandermeer JH, Boucher DH (1978) Varieties of mutualistic interaction in population models. Journal of Theoretical Biology 74(4): 549–558. https://doi.org/10.1016/0022-5193(78)90241-2

Varón EH, Eigenbrode SD, Bosque-Pérez NA, Hilje L (2007) Effect of farm diversity on harvesting of coffee leaves by the leaf-cutting ant *Atta cephalotes*. Agricultural and Forest Entomology 9(1): 47–55. https://doi.org/10.1111/j.1461-9563.2006.00320.x

Weber NA (1966) Fungus-growing ants. Science 153(3736): 587–604. https://doi.org/10.1126/science.153.3736.587

Wilson EO (1980) Caste and division of labor in leaf-cutter ants (Hymenoptera: Formicidae: Atta): II. The ergonomic optimization of leaf cutting. Behavioral Ecology and Sociobiology 7(2): 157–165. http://www.jstor.org/stable/4599320

Zanetti R, Zanuncio JC, Vilela EF, Leite HG, Jaffé K, Oliveira AC (2003) Level of economic damage for leaf-cutting ants (Hymenoptera: Formicidae) in *Eucalyptus* plantations in Brazil. Sociobiology 42(2): 433–442.

Supplementary material 1

Original grooming data

Authors: Isabella Bueno, João Vitor Mendes Ferraz de Toledo, Lucas Santos Canuto, Wesley Augusto Conde Godoy

Data type: xlsx

Explanation note: Laboratory estimates of the time spent on self-grooming and allogrooming for the control, *M. anisopliae*, and *E. phialicopiosa* treatments.

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/biorisk.23.151538.suppl1